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(71) Applicant: BRISTOL-MYERS SQUIBB COMPANY [US/US]; P.O. Box 4000, Princeton, NJ 08543-4000 (US).

(72) Inventors: BLANAR, Michael, A.; 1325 Summerhill Drive, Malvern, PA 19355 (US). DWORETZKY, Steven; 43 High Meadow Lane, Middlefield, CT 06455 (US). YANG, Wen-Pin; 25 Rutgers Lane, Princeton, NJ 08540 (US). LEVESQUE, Paul, C.; 1925 Westover Road, Yardley, PA 19067 (US). GRIBKOFF, Valentin, K.; 142 Williams Road, Wallingford, CT 06492 (US). NEUBAUER, Michael, G.; 7 Cypress Point Court, Skillman, NJ 08558 (US). LITTLE, Wayne, A.; 1206 Chestershire Place, Pottstown, PA 19465 (US).

(74) Agents: KLEIN, Christopher, A. et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, NJ 08543-4000 (US).

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(54) Title: KCNQ POTASSIUM CHANNELS AND METHODS OF MODULATING SAME

(57) Abstract

The present invention relates to KCNQ proteins defining potassium channels. In particular, the invention concerns the human KCNQ2, human KCNQ3, murine KCNQ2, and rat KCNQ2 proteins reported herein. KCNQ2 and KCNQ3 proteins are nervous system-selective and may be involved in neurotransmission and neuroprotection. The KCNQ2 and KCNQ3 of the present invention can be used to assay for modulators of the proteins, which would be useful in treatment of such disorders as ataxia, myokymia, seizures, Alzheimer's disease, Parkinson's disease, age-associated memory loss, learning deficiencies, motor neuron diseases, epilepsy, stroke, and the like.

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KCNQ POTASSIUM CHANNELS AND METHODS OF MODULATING SAME

Field of the Invention

The present invention concerns nucleic acids and proteins for potassium channels, as well as related vectors, host cells, processes for preparation, and methods of use. Included within the present invention are methods of screening for compounds that bind to and/or otherwise modulate the potassium channel proteins disclosed herein. Additionally, the present invention encompasses methods of modulating the potassium channels disclosed herein, for example methods of opening/activating or closing/inactivating said potassium channels.

Background of the Invention

Among ion channels, potassium ion (K^+) channels are the most ubiquitous and diverse. They include three major structural classes - channels with six, four, or two transmembrane domains. The six transmembrane domain potassium channels are divided further into different families, such as Shaker-like, eag-like and Slo-like potassium channels. Recent identification of KvLQT1 established a new family of six-transmembrane potassium channels. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetti et al. (1996) Nature 384: 80-83; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94: 4017-22; Wang et al. (1996) Nature Genetics 12: 17-23. Search of DNA and protein sequence databanks reveals additional potential members of KvLQT1-related channels in C. elegans as well as in the human. Wei et al. (1996), Neuropharmacology 35: 805-29; ; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94: 4017-2.

One or more types of K^+ channels reside on cell membranes where they are remarkably selective for K^+ over other ions. In excitable cells, K^+ channels modulate action potential configuration. Efflux of potassium is the major mechanism for repolarization, maintenance, and hyperpolarization of resting membrane potential. Halliwell (1990) in Potassium channels-structure, classification, function and therapeutic potential (N. S. Cook, ed.); 348-381; Jan, L. Y. and Jan, Y. N. (1992), Ann. Rev. Physiol. 54: 537-55; Pongs (1992), Physiol. Rev. 72: S69-S88.

In neurons, K^+ channels regulate neuronal excitability, action potential shape and firing pattern, and neurotransmitter release. These channels can be gated by various stimuli, such as intracellular second messengers, membrane potential, ions, and neurotransmitters. Hille (1992), Ionic channels of excitable membranes; Catterall (1995), Ann. Rev. Biochem. 64: 493-531. Neuronal K^+ channels are critical to such neuronal functions as neurotransmission and neuroprotection, and they may affect perception, learning, behavior, and the like.

Recently, the nomenclature for KvLQT1 and the KvLQT1-related channels was changed. Biervert et al. (1998), Science 279:403-406. KvLQT1 was re-named KCNQ1, and the KvLQT1-related channels (KvLR1 and KvLR2) were re-named as KCNQ2 and KCNQ3, respectively. Therefore, throughout this specification, reference to KCNQ1 is equivalent to KvLQT1; reference to KCNQ2 is equivalent to KvLR1; and reference to KCNQ3 is equivalent to KvLR2.

Benign familial neonatal convulsions ("BFNC"), a class of idiopathic generalized epilepsy, is an autosomal-dominantly inherited disorder of newborns. BFNC has recently been linked to mutations in two putative K^+ channel genes, KCNQ2 and KCNQ3. Biervert et al., supra; Charlier et al. (1998), Nature Genetics 18:53-55; Singh et al. (1998) Nature Genetics 18:25-29. Preliminary functional characterization of KCNQ2 confirmed that this gene encodes a voltage-activated K^+ channel. Singh et al., supra.

Summary of the Invention

The present invention discloses novel nervous system-specific potassium channels referred to herein as KCNQ2 (formerly called KvLR1) and KCNQ3 (formerly called KvLR2). Within the present invention are human KCNQ2 (Figure 2), human KCNQ3 (Figure 23), murine KCNQ2 (Figure 10), and rat KCNQ2 (Figure 16 and Figure 17). The invention encompasses the amino acid sequences of these proteins and the nucleic acid sequences encoding said proteins, as well as variations in the nucleic acid sequences due to degeneracy in the genetic code.

The present invention provides for nucleic acid molecules at least about 70% identical to the consensus sequence of the nucleotide sequences disclosed herein. Preferably, the present invention provides: (a) a purified and isolated nucleic acid molecule encoding a KCNQ2 and/or KCNQ3 protein of the present invention; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 70% sequence identity, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a); or (d) a fragment of (a) or (b) which will hybridize to (a) or (b) under stringent conditions, said fragment preferably comprising at least 15 nucleotides. Preferred nucleic acid sequences encoding the KCNQ2 and KCNQ3 proteins of the present invention are found in SEQ ID NO:3, SEQ ID NO:17, SEQ ID NO:7 and SEQ ID NO:5.

Also within the scope of the present invention are amino acid sequences at least about 70% identical to the consensus sequence of the proteins disclosed herein. Preferably, the invention covers: (a) amino acid sequences comprising the KCNQ2 and/or KCNQ3 proteins of the present invention; and (b) amino acid sequences having at least 70% sequence identity, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a). Preferred amino acid sequences comprising the KCNQ2 and KCNQ3 proteins of the present invention are found in SEQ ID NO:4, SEQ ID NO:18, SEQ ID NO:8 and SEQ ID NO:6.

The invention further concerns novel nucleic acids and associated vectors, host cells, and methods of use. Preferably, the nucleic acid molecule is a DNA molecule. Further preferred are nucleotide sequences encoding the amino acid sequences of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:18 as well as proteins about 70% or more identical to these sequences. Also preferred are nucleotide sequences about 80% or more identical to SEQ ID NO:1; most preferred are SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:17.

The invention further concerns nucleic acids obtained by PCR with degenerate oligonucleotide primers. Persons of ordinary skill in the art could devise such primers

based on the consensus sequence described herein. PCR techniques are described in White et al. (1989), Trends Genet. 5: 185-9.

This invention further concerns nucleic acid vectors comprising a nucleic acid sequence coding for a KvLR/KCNQ protein, host cells containing such vectors, and polypeptides comprising the amino acid sequence of a KvLR/KCNQ protein. Preferably, the vector encodes a full-length KvLR/KCNQ protein and the polypeptide is full-length KvLR/KCNQ protein. The inventors prefer frog expression vectors such as pSP64T or derivatives thereof (Melton et al. (1984), Nucl. Acids Res. 12: 7057-70); mammalian cell expression vectors such as pcDNA3 (available from Invitrogen); or bacterial cell expression vectors such as pET-30 (available from Novagen or Promega).

This invention further concerns host cells transformed with the above-described vectors. The inventors prefer Xenopus oocytes, mammalian cells (e.g., HEK293, CHO, L929), and bacterial cells (e.g., E. coli, especially BL21(DE3), available from Novagen). The inventors particularly prefer the cells deposited as ATCC Acc. No. CRL-1573 (American Type Culture Collection, 10801 University Boulevard, Manassas VA 20110-2209).

The invention also concerns methods for detecting nucleic acids coding for KCNQ/KvLR proteins and processes for detecting molecules that bind to and/or otherwise modulate the activity of KCNQ/KvLR proteins. "Modulate" encompasses both channel openers/activators and channel closers/inactivators.

The invention also concerns methods of modulating the KCNQ proteins, specifically methods of opening/activating or closing/inactivating the KCNQ2 and/or KCNQ3 channels. Additionally, the present invention encompasses a method of treating disease by modulating the activity of the KCNQ proteins.

All references cited herein, whether *supra* or *infra*, are hereby incorporated herein in their entirety.

Description of the Drawings

Figure 1 shows isolation of a full-length human KCNQ2/KvLR1 cDNA. A full-length human KCNQ2/KvLR1 cDNA was derived from two overlapping cDNA

clones. "S1" through "S6" signify transmembrane domains 1 through 6; "H5" signifies the pore-forming domain (this domain is also referred to herein as the "P" domain); ORF, the open-reading frame; 3' UTR, the 3' untranslated regions. The locations of various EST clones and probes also are shown. (The figure is not drawn to scale.)

Figures 2A and 2B show the nucleotide and deduced amino acid sequence of human KCNQ2/KvLR1.

Figure 3 shows a sequence comparison of human KCNQ2/KvLR1 and human KCNQ1/KvLQT1. "|" denotes amino acid sequence identity. The C-terminal amino acids of both proteins are not shown.

Figure 4 shows a sequence comparison of human KCNQ3/KvLR2 and human KCNQ1/KvLQT1. "|" denotes amino acid sequence identity. The C-terminal amino acids of both proteins are not shown. The N terminal amino acids of KCNQ3/KvLR2 are not shown.

Figure 5 shows expression of KCNQ2/KvLR1 and KCNQ3/KvLR2 in human tissues and various portions of human brain. Figure 5A shows KCNQ2/KvLR1. Figure 5B shows KCNQ3/KvLR2. Poly(A+) mRNA Northern blots were hybridized individually to radiolabeled KCNQ2-specific (Figure 5A) or KCNQ3-specific (Figure 5B) probes. RNA molecular weight markers are indicated on the left.

Figure 6 shows functional characterization of KCNQ2/KvLR1 currents in Xenopus oocytes.

In Figures 6A and 6B, families of currents from water-injected (Figure 6A) and human KCNQ2/KvLR1 cRNA-injected (Figure 6B) oocytes were elicited by 1 second voltage steps, from a holding potential of -80 mV, to test potentials ranging from –100 to +40 mV in 10 mV increments.

Figure 6C shows the peak current-voltage (I-V) relationship for oocytes expressing human KCNQ2/KvLR1. Currents were recorded using the protocol described above for Figures 6A and 6B.

Figure 6D shows dependence of tail current reversal potential (E_{rev}) on the external K^+ concentration. Tail currents were elicited at potentials of –110 to +10 mV following a 1 second pulse to +20 mV (n = 6 oocytes) while the external K^+

concentration was varied between 2, 10, 40, and 98 mM. E_{rev} under each condition was determined for each oocyte by measuring the zero intercept from a plot of tail current amplitude versus test potential. The dashed line has a slope of 58 mV and is drawn according to the Nernst equation for a perfectly selective K^+ channel. Data are the mean ± SEM from six experiments.

Figure 7 shows pharmacologic characterization of KCNQ2/KvLR1 currents in Xenopus oocytes. In particular, this figure shows effects of E-4031, 4-AP, TEA, charybdotoxin and clofilium on human KCNQ2/KvLR1 current. Superimposed currents were recorded during 1 second steps to +30 mV, from -80 mV, during the same experiment. Compounds were applied via bath perfusion in order from top to bottom. The bath was perfused with control solution for 5 minutes, or until effects reversed completely, between compounds. Similar results were obtained in three additional oocytes.

Figure 8 shows co-expression of minK and human KCNQ2/KvLR1 in Xenopus oocytes.

Figure 8A shows the effect of 1 mM TEA on membrane currents recorded from an oocyte injected with human KCNQ2/KvLR1 alone. Superimposed currents were recorded during 1 second voltage steps to +40 mV from a holding potential of -80 mV before and after applying TEA via the bath. TEA reduced human KCNQ2/KvLR1 current by over 80%.

Figure 8B shows the effect of 1 mM TEA on membrane currents recorded from an oocyte injected with minK and human KCNQ2/KvLR1. Currents were elicited using the protocol in Figure 8A. TEA partially inhibited minK + human KCNQ2/KvLR1 currents, however, the amplitude and kinetics of the TEA-insensitive current component were similar to currents observed in oocytes injected with minK alone.

Figure 9 shows murine KCNQ2/KvLR1 expression in the brain of adult mouse. Figure 9A is a dark-field photograph from a coronal section through an adult mouse brain hybridized with a radiolabeled antisense KCNQ2/KvLR1 probe, showing KCNQ2/KvLR1 transcripts in the pyramidal cell layers of the hippocampus. Lower levels of expression were detected in the granular cell layer of the dentate gyrus.

Figure 9B is a dark-field photograph from a similar region as shown in Figure 9A, but hybridized with a sense probe; little KvLR1-specific expression was detected with this probe. Magnification: 125x for both Figure 9A and Figure 9B.

Figure 9C shows partial murine KCNQ2/KvLR1 nucleotide sequence (SEQ ID NO:9) and amino acid sequence (SEQ ID NO:10). This sequence was obtained through PCR amplification of a mouse brain cDNA library using the oligonucleotides MABms 278 (SEQ ID NO:11) and MABms 315 (SEQ ID NO:12) based on the human KCNQ2/KvLR1 sequence. The PCR fragments were isolated, subcloned, and sequenced. A 226 bp fragment as shown above was used in a probe for in situ hybridization. The nucleotide sequence is 80% identical to human KCNQ2/KvLR1 (96% identity in amino acid sequence).

MABms 278 (SEQ ID NO:11):

5'-GGCCGAATTCTGTTTCTCAGCAGCTCCAGC-3'

MABms 315 (SEQ ID NO:12):

5'-GCGCGAATTCGAGCAGCACAGGCA(A/G)AA(A/G)CA-3'

Figure 10A through Figure 10D show the DNA and translated amino acid sequence of the mouse brain KCNQ2/KvLR1 gene. Figure 10E shows hydropathy analysis of the mouse brain KCNQ2/KvLR1 gene. The hydropathy plot reveals the pattern typical of voltage-sensitive K^+ channels with 6 putative membrane spanning domains (S1-S6) and a pore region (P).

Figure 11 shows sequence alignment of the mouse heart KCNQ1/K vLQT1 and mouse brain KCNQ2/KvLR1 potassium channels. The alignment of these two genes shows a 40% overall amino acid identity (indicated by the shaded areas) and 62.5% identity within the spanning and pore domains. Putative membrane spanning and pore domains are indicated by the boxes. The signature sequence for a potassium channel, GYG, is observed within the pore region and the voltage sensor, RXXQXXRXXR, is within the S4 domain.

Figure 12 shows alternative splice exons in the 3' end of murine KCNQ2/KvLR1. At least two splice exons, that when translated give the amino acid sequences shown in A and B (SEQ. ID. NOS.: 13 and 14), have been identified in the murine KCNQ2/KvLR1 gene at amino acid positions 372 and 406, respectively.

Figure 13 shows a mouse multiple tissue northern blot. A northern blot was probed with a fragment of the mouse KCNQ2/KvLR1 gene (nucleotides 1140-2306). A single transcript of 8.2 kb is observed in brain, but not is seen in other tissues.

Figure 14 shows in situ hybridization of rat brain. The composite shows three regions where the rat KCNQ2/KvLR1 message is strongly expressed. The antisense probes show strong signal in the hippocampus, dentate gyrus, cortex, and motor nucleus of the trigeminal nerve. Sense probe controls show little background.

Figure 15 shows electrophysiology characterization of mouse KCNQ2/KvLR1-mediated whole-cell currents expressed in Xenopus oocytes. In Figure 15A, 10 mV step depolarizations from a -80 to +40 produced a family of outward currents that were significantly different than control cells. Addition of 1 mM TEA blocked the KvLR1-mediated currents and background chloride currents were not affected by TEA. Clofilium, a blocker of heart I_{Ks} and I_{Kr} currents, was shown to partially block the KCNQ2/KvLR1- mediated currents when depolarized to from -80 to +40 μV. Figure 15B shows uninjected controls.

Figure 16 shows an alignment of the consensus nucleotide sequence (SEQ ID NO:1) and the nucleotide sequences of the human KCNQ3/KvLR2 (SEQ ID NO:17), human KCNQ2/KvLR1 (SEQ ID NO:3), mouse KCNQ2/KvLR1 (SEQ ID NO:5), and rat KCNQ2/KvLR1 (SEQ ID NO:7). "|" denotes sequence identity; "–" represents non-consensus sequence; and "*" denotes a space introduced to optimize sequence identity.

Figure 17 shows an alignment of the consensus amino acid sequence (SEQ ID NO:2) and the amino acid sequences of the human KCNQ3/KvLR2 (SEQ ID NO:18), human KCNQ2/KvLR1 (SEQ ID NO:4), mouse KCNQ2/KvLR1 (SEQ ID NO:6), and rat KCNQ2/KvLR1 (SEQ ID NO:8) proteins. As in Figure 16, "|" denotes sequence identity; "–" represents non-consensus sequence; and "*" denotes a space introduced to optimize sequence identity.

Figure 18 shows the functional characterization of KCNQ3 currents. Figure 18A shows families of currents from KCNQ3 cRNA-injected oocytes elicited by 1 sec voltage steps, from a holding potential of -80 mV, to test potentials ranging from -70 to +50 mV in 10 mV increments. Figure 18B shows I-V relationship for oocytes

expressing KCNQ3 (n=6). Currents were recorded using the protocol in Figure 18A. Figure 18C shows dependence of tail current E_{rev} on the external K^+ concentration. The line has a slope predicted by the Nernst equation for a perfectly selective K^+ channel. Each value is the mean±SEM from 6 oocytes. Figure 18D shows effects of E-4031, 4-AP, TEA and clofilium on KCNQ3 current. Superimposed currents were recorded during 1 sec steps to +20 mV, from -80 mV, during the same experiment. Compounds were applied via bath perfusion in order from top to bottom. The bath was perfused with control solution for 5 min, or until effects reversed completely, between compounds. Similar results were obtained in three additional oocytes.

Figure 19 shows co-expression of KCNQ2 and KCNQ3. Figure 19A shows families of currents from KCNQ2, Figure 19B from KCNQ3, and Figure 19C from KCNQ2+KCNQ3 cRNA-injected oocytes elicited by 1 sec voltage steps, from a holding potential of -80 mV, to test potentials ranging from -70 to +50 mV (10 mV increments). Figure 19D shows current-voltage (I-V) relationship for oocytes expressing KCNQ2+KCNQ3 (n=6). Currents were recorded using the protocol in Figures 19A-19C. Figure 19E shows dependence of tail current reversal potential (Erev) on the external K^+ concentration. The dashed line has a slope predicted by the Nernst equation for a perfectly selective K^+ channel. Each value is the mean±SEM from 6 oocytes. Figure 19F shows the effects of 4-AP, TEA, charybdotoxin and clofilium on KCNQ2+KCNQ3 current. Superimposed currents were recorded during 1 sec steps to +20 mV, from -80 mV, during the same experiment. Compounds were applied via bath perfusion in order from top to bottom. Similar results were obtained in 4 additional oocytes.

Figure 20 shows the interaction of KCNE1 (minK) with KCNQ2+KCNQ3 currents. Families of currents from KCNE1 (Figure 20A), KCNQ2+KCNQ3 (Figure 20B) and KCNQ2+KCNQ3+KCNE1 (Figure 20C) cRNA-injected oocytes elicited by 1 sec voltage steps, from a holding potential of -80 mV, to test potentials ranging from -70 to +50 mV (10 mV increments). Inset of Figure 20A shows KCNE1 currents elicited by 5 sec voltage steps from -80 mV to potentials ranging from -30 to +50 mV (20 mV increments) in the same oocyte.

Figure 21 is a photograph of in situ hybridization with rat KCNQ2 showing a cross section of the rat spinal cord. Figure 21(A) is under low magnification (55x); several areas can be visualized with a relatively high signal. Figure 21(B) is under higher magnification (322x); each high signal area is one cell and they appear by their size to be motoneurons.

Figure 22A shows macroscopic murine KCNQ2 current recorded from inside-out membrane patch excised from a CHO cell stably expressing murine KCNQ2. The current displays slow activation and outward rectification. Figure 22B shows patch clamp recording of single channel currents in an excised inside-out patch from a CHO/murine KCNQ2 cell. There are at least 2 channels in the patch; single channel conductance of KCNQ2 was estimated to be between 24 and 30 pS. All recordings were made in symmetrical 140 mM K^+ using standard techniques.

Figure 23 shows the nucleotide and deduced amino acid sequence of human KCNQ3 (also referred to herein as KvLR2).

Detailed Description of the Invention

The following definitions apply to the terms used throughout this specification, unless otherwise defined in specific instances:

"cloning" - isolation of a particular gene from genetic material, for example a genome, genomic library, or cDNA library into a plasmid or other vector;

"KvLR protein" - a protein having at least about 70% identity with the consensus sequence (SEQ. ID. NO.: 2). It may also be referred to as a "KCNQ protein", "KvLR/KCNQ protein" or "KCNQ/KvLR protein".

"KCNQ1" - the protein formerly known as KvLQT1.

"KCNQ2" - the protein formerly known as KvLR1.

"KCNQ3" - the protein formerly known as KvLR2.

"stringent conditions " (as used concerning nucleic acid hybridization)—For example, Southern blotting washed in 1 X SSC and 0.1% SDS at a temperature of at least about 42 °C. For additional stringent conditions, see Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).

"multi-copy plasmid"—a plasmid having greater than one copy present in a cell (typically 10 to 30 copies);

"Northern blotting"—a method of identifying particular RNA fragments by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide;

"open reading frame" or "ORF"—a DNA sequence containing a series of nucleotide triplets coding for amino acids and lacking any termination codes;

"plasmid"—cytoplasmic, autonomously replicating DNA elements found in microorganisms;

"promoter"—a region on DNA at which RNA polymerase binds and initiates transcription; and

"Southern blotting"—a method of identifying particular DNA fragments by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide.

For definitions of other terms in this specification, see F. Sherman et al., Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1987) and Lewin, B., Genes IV, Oxford University Press, Oxford (1990).

The following definitions apply to abbreviations in this specification, unless otherwise defined in specific instances:

BFNC	benign familial neonatal convulsions
BLAST	basic local alignment search tool
CHO	Chinese hamster ovary cells
DTT	dithiothreitol
DRG	dorsal root ganglion
EDTA	ethylene diamino tetraacetic acid
EST	expressed sequence tags
GPCR	G-protein–coupled receptor
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline

PCR	polymerase chain reaction
SDS	sodium dodecyl sulfate
SSC	buffer containing 150 mM NaCl, 15 mM Na_3citrate • 2 H_2O, pH 7.0.
TEA	tetraethylammonium

For additional abbreviations, see Aldrichimica Acta, Vol. 17, No. 1 (1984).

Use and utility

It is believed by those skilled in the art that KCNQ proteins may be involved in neurotransmission. Persons of ordinary skill in the art can use KCNQ/KvLR proteins of the present invention to assay for modulators of KCNQs/KvLRs. KCNQ modulators would be useful in treatment of such disorders as ataxia, myokymia, seizures (e.g., epileptic seizures), Alzheimer's disease, Parkinson's disease, age-associated memory loss, learning deficiencies, motor neuron diseases, stroke, and the like.

Because KCNQ2 and KCNQ3 are nervous system-selective potassium channels, drug specificity is built into any KCNQ2/KCNQ3-specific modulator. A drug specific for KCNQ2 and/or KCNQ3 protein would thus avoid side-effects on peripheral tissues that contain potassium channels. Significantly, KCNQ2/KCNQ3-specific modulators would avoid side-effects on the heart, which contains numerous types of potassium channels.

The KCNQ nucleic acids of the present invention, or antisense nucleic acids, may be useful therapeutic or diagnostic agents. For such gene therapy, the nucleic acids may be incorporated into vectors and/or formulated as described below and in further detail in the art.

Persons skilled in the art can use the polypeptides and nucleic acids of this invention to prepare vectors, cells or cell lines, and antibodies. All of these are useful in assays for identification of KCNQ2/KCNQ3 protein modulators.

One can administer KCNQ2 and/or KCNQ3 protein modulators to various mammalian species, such as monkeys, dogs, cats, mice, rats, humans, etc. By known methods, persons skilled in the pharmaceutical art can incorporate KCNQ2/KCNQ3 protein modulators in a conventional systemic dosage form, such as a tablet, capsule,

elixir or injectable formulation. The above dosage forms will also include any necessary physiologically acceptable carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid or sodium bisulfite) or the like.

Process of preparation

In general

This specification describes the cloning and functional expression of full-length human cDNA clones of KCNQ2 (KvLR1) and KCNQ3 (KvLR2), preferably the human KCNQ2 nucleic acid sequence (Figure 2) as shown in SEQ ID NO:3, the human KCNQ2 amino acid sequence (Figure 2) as shown in SEQ ID NO:4, the human KCNQ3 nucleic acid sequence (Figure 23) as shown in SEQ ID NO:17, and the human KCNQ3 amino acid sequence (Figure 23) as shown in SEQ ID NO:18. Also disclosed is a full-length murine cDNA clone of KCNQ2 (murine KvLR1; Figure 10), preferably the murine KCNQ2 nucleic acid sequence as shown in SEQ ID NO:5, and the murine KCNQ2 amino acid sequence as shown in SEQ ID NO:6. Additionally, the present invention covers a rat KCNQ2 sequence (Figure 16 and Figure 17), preferably the rat KCNQ2 nucleic acid sequence as shown in SEQ ID NO:7, and the rat KCNQ2 amino acid sequence as shown in SEQ ID NO:8. The gating kinetics and macroscopic current properties of human, murine and rat KCNQ2 and KCNQ3 currents are similar to those of KCNQ1. However, KCNQ2 and KCNQ3 are specifically localized within the nervous system and have different pharmacological properties.

DNA clones comprising nucleotide sequences encoding the following KCNQ2 and KCNQ3 proteins of the present invention were deposited with the American Type Culture Collection ("ATCC") (10801 University Blvd., Manassas, VA 20110-2209) on _____, 1998: human KCNQ2, ATCC Accession Number _____; human KCNQ3, ATCC Accession Number _____; and murine KCNQ2, ATCC Accession Number _____. The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are

not an admission that a deposit is required under 35 U.S.C. 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

Nucleic acids

With the human KCNQ2, human KCNQ3, murine KCNQ2, and rat KCNQ2 gene sequences in hand, one skilled in the art can obtain KCNQ nucleic acids of this invention by known methods. Such methods include: (1) Southern and Northern blotting; (2) Western immunoblotting; (3) chemical synthesis; (4) synthesis by polymerase chain reaction (PCR) from primers; (5) expression cloning; and (6) subtractive cDNA cloning.

Persons skilled in the art can also modify the nucleic acids coding for the KCNQ proteins of the present invention to prepare useful mutations. For example, one may modify the sequence to provide additional restriction endonuclease recognition sites in the nucleic acid. Such mutations may be silent or may change the amino acid encoded by the mutated codon. One can prepare these modified nucleic acids, for example, by mutating the nucleic acid coding for KCNQ2 to result in deletion, substitution, insertion, inversion or addition of one or more amino acids in the encoded polypeptide. For methods of site-directed mutagenesis, see Taylor, J. W. et al. (1985), Nucl. Acids Res. 13, 8749-64 and Kunkel, J. A. (1985), Proc. Natl. Acad. Sci. USA 82: 482-92. In addition, kits for site-directed mutagenesis are available from commercial vendors (e.g., BioRad Laboratories, Richmond, CA; Amersham Corp., Arlington Heights, IL). For disruption, deletion and truncation methods, see Sayers, J. R. et al. (1988), Nucl. Acids Res. 16: 791-800.

This invention also comprises modified nucleic acids, including (1) alternative splice exon variants; (2) allelic variants; and (3) chimeric channels in which the fusion construct comprises a KCNQ modulatory site. Such modified nucleic acids can be obtained by persons of ordinary skill in the art when armed with the present disclosure.

Expression vectors

This invention further concerns expression vectors comprising a nucleotide sequence encoding a KCNQ protein of the present invention. Preferably, the expression vectors comprise all or a portion of the nucleic acid sequence as shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:17.

Expression vectors are usually plasmids, but the invention includes other vector forms that serve equivalent functions and become known in the art subsequently hereto. A person skilled in the art might also stably integrate a sequence encoding a KCNQ protein into the chromosome of an appropriate host cell.

Expression vectors typically contain regulatory elements capable of affecting expression of a KCNQ protein. These regulatory elements can be heterologous or native KCNQ elements. Typically, a vector contains an origin of replication, a promoter, and a transcription termination sequence. The vector may also include other regulatory sequences, including mRNA stability sequences, which provide for stability of the expression product; secretory leader sequences, which provide for secretion of the expression product; environmental feedback sequences, which allow expression of the structural gene to be modulated (e.g., by the presence or absence of nutrients or other inducers in the growth medium); marking sequences, which are capable of providing phenotypic selection in transformed host cells; restriction sites, which provide sites for cleavage by restriction endonucleases; and sequences which allow expression in various types of hosts, including prokaryotes, yeasts, fungi, plants and higher eukaryotes.

An expression vector of this invention is at least capable of directing the replication, and preferably the expression, of the nucleic acids and protein of this invention. Suitable origins of replication include, for example, the Col E1, the SV4O viral and the M13 origins of replication. Suitable promoters include, for example, the cytomegalovirus promoter, the lacZ promoter, the gal10 promoter and the Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) polyhedral promoter. Suitable termination sequences include, for example, the bovine growth hormone, SV40, lacZ and AcMNPV polyhedral polyadenylation signals. Examples of

selectable markers include neomycin, ampicillin, and hygromycin resistance and the like.

Persons skilled in the art may insert DNA encoding a KCNQ protein of the present invention into several commercially available vectors. Examples include vectors compatible with mammalian cells, such as pcDNA3 or pCEP4; baculovirus vectors such as pBlueBac; prokaryotic vectors such as pcDNA2; and yeast vectors such as pYes2. For vector modification techniques, see Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Host cells

This invention additionally concerns host cells containing an expression vector that comprises a sequence encoding a KCNQ protein, preferably the KCNQ2 and/or KCNQ3 proteins of the present invention. The host cells preferably contain an expression vector which comprises all or part of the DNA sequence having the nucleotide sequence substantially as shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:17, particularly the coding regions thereof. Suitable host cells include both prokaryotic cells (e.g., E. coli strains HB101, DH5a, XL1 Blue, Y1090 and JM101) and eukaryotic cells (e.g., Spodoptera frugiperda insect cells, CHO cells, COS-7 cells, HEK 293 cells, human skin fibroblasts, and S. cerevisiae cells).

Persons skilled in the art may introduce expression vectors into host cells by various methods known in the art. Exemplary methods are transfection by calcium phosphate precipitation, electroporation, liposomal fusion, nuclear injection, and viral or phage infection. One may then culture the host cell under conditions permitting expression of large amounts of KCNQ protein.

One may identify such modified host cells by any of six general approaches:

(a) DNA-DNA hybridization with probes complementary to the sequence encoding KCNQ protein (Southern blotting).

(b) detection of marker gene functions, such as thymidine kinase activity, resistance to antibiotics, and the like. A marker gene can be placed in the same

plasmid as the KCNQ sequence under the regulation of the same or a different promoter.

(c) detection of mRNA transcripts by hybridization assays (e.g., Northern blotting or a nuclease protection assay using a probe complementary to the RNA sequence).

(d) immunodetection of gene expression (e.g., by Western blotting with antibody to KCNQ protein).

(e) detection of potassium channel activity, such as by patch-clamp analysis, radioisotope (e.g., ^{86}Rb) efflux, or membrane potential-sensitive reagents (e.g., Dibac from Molecular Probes International).

(f) PCR with primers homologous to expression vector sequences or sequences encoding KCNQ protein. The PCR produces a DNA fragment of predicted length, indicating incorporation of the expression system in the host cell.

Persons skilled in the art may determine DNA sequences by various known methods. See, for example, the dideoxy chain termination method in Sanger et al. (1977), Proc. Natl. Acad. Sci. USA 74: 5463-7 and the Maxam-Gilbert method in Maxam-Gilbert (1977), Proc. Natl. Acad. Sci. USA 74: 560-4.

One may use the host cells of this invention in a variety of ways that are now apparent. One may use the cells to screen for compounds that bind to or otherwise modulate or regulate the function of KCNQ protein, which would be useful for modulation, for example activation, of KCNQ2 and/or KCNQ3 protein activity; to study signal transduction mechanisms and protein-protein interactions; and to prepare KCNQ protein for the uses described below.

Not all expression vectors and DNA regulatory sequences will function equally well to express the DNA sequences of this invention. Neither will all host cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells using the guidance provided herein without undue experimentation and without departing from the scope of the invention.

Polypeptides

This invention further concerns polypeptides comprising all or a portion of the amino acid sequences of a KCNQ2 and/or KCNQ3 protein. The inventors prefer polypeptides comprising all or a portion of the amino acid sequences shown as in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:18. Where a portion of the KCNQ2 and/or KCNQ3 protein is used, preferably the portion exhibits K^+ channel activity or can be modulated to exhibit K^+ channel activity. For example, and within the scope of the invention, are polypeptides that comprise all or a portion of KCNQ2 and/or KCNQ3 that may contain one or more mutations so that the protein(s) fails to exhibit K^+ channel activity, but that can be used to screen for compounds that will activate the protein or portion thereof.

Persons having ordinary skill in the art may prepare these polypeptides by methods known in the art. For example, one may use chemical synthesis, such as the solid phase procedure described by Houghton et al. (1985), Proc. Natl. Acad. Sci. 82: 5131-5. Another method is in vitro translation of mRNA. One may also produce the polypeptides in the above-described host cells, which is the preferred method. For example, one may synthesize DNA comprising all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:17 by PCR as described above, insert the synthesized DNA into an expression vector, transform a host cell with the expression vector, and culture the host cell to produce the desired polypeptides.

Persons skilled in the art can isolate and purify such polypeptides by any one of several known techniques; for example, ion exchange chromatography, gel filtration chromatography and affinity chromatography. Such techniques may require modification of the protein. For example, one may add a histidine tag to the protein to enable purification on a nickel column.

Persons skilled in the art can use the polypeptides of the invention in a wide variety of ways. For example, one may use them to generate polyclonal or monoclonal antibodies. One may then use such antibodies for immunodetection (e.g., radioimmunoassay, enzyme immunoassay, or immunocytochemistry), immunopurification (e.g., affinity chromatography) of polypeptides from various sources, or immunotherapy (i.e., for potassium channel inhibition or activation).

Persons skilled in the art may make modified KCNQ polypeptides by known techniques. Such modifications may cause higher or lower activity, permit higher levels of protein production, or simplify purification of the protein. Such modifications may help identify specific KCNQ2 and/or KCNQ3 amino acids involved in binding, which in turn may help rational drug design of KCNQ2/KCNQ3 modulators. One can make amino acid substitutions based on similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine, glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. All such modified polypeptides are included within the scope of the invention.

The inventors contemplate a number of other variations of the above-described polypeptides. Such variations include salts and esters of the polypeptides, as well as precursors of the aforementioned polypeptides (e.g., having N-terminal substituents such as methionine, N-formylmethionine and leader sequences). The invention includes all such variations.

Method for detecting nucleic acids

The present invention further concerns a method for detecting nucleic acids encoding KCNQ protein. In this method, a person of ordinary skill in the art (a) contacts nucleic acids of unknown sequence with a nucleic acid having a sequence complementary to a known coding sequence (e.g., a sequence of at least about 10 nucleotides from, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:17, particularly the coding regions thereof), wherein the latter nucleic acid has a detectable marker; and (b) determines the presence of marker bound to any of the nucleic acids of unknown sequence. The presence of bound marker indicates the presence of the desired nucleic acids. One can apply this method to detect KCNQ nucleic acids from other tissues (which may have different regulatory elements) and nucleic acids from other species (e.g., monkey).

Persons of ordinary skill in the art generally know how to obtain nucleic acids to be analyzed in this method. For genomic DNA, one can rapidly freeze tissue, crush the tissue into readily digestible pieces, and incubate the crushed tissue in proteinase K and SDS to degrade most cellular proteins. One can then deproteinize the genomic DNA by successive phenol/chloroform/isoamyl alcohol extractions, recover DNA by ethanol precipitation, dry it and resuspend it in buffer. For RNA, one can lyse cultured cells in 4M guanidinium solution, draw the lysate through a 20-gauge needle, pellet the RNA through a cesium chloride step gradient, and remove the supernatant. The pellet should contain purified RNA.

The detectable marker may be a radioactive ion linked to one of the nucleotides of the complementary nucleic acid. Common radioactive labels are ^{32}P and ^{35}S, although one may also use other labels such as biotin. Persons skilled in the art are aware of various methods to attach the labels to the complementary nucleic acid (e.g., the random primer method for attachment of ^{32}P or ^{35}S).

Persons of ordinary skill in the art generally know how to carry out such a method of detecting nucleic acids. For example, one may perform a Southern or northern blot using a radiolabeled KCNQ complementary oligonucleotide probe. One can then detect hybridization by autoradiography. Depending on the marker, one may also use other detection methods (e.g., spectrophotometry).

Methods for detecting KCNQ2/KCNQ3 protein modulators

This invention further concerns methods for detecting modulators of the KCNQ2 and/or KCNQ3 proteins of the present invention. A screen for KCNQ protein modulators entails detecting binding of molecules (e.g., polypeptides, natural products, synthetic compounds) in cells expressing KCNQ protein.

Cloning and sequencing of KCNQ protein enables construction of cells useful in screening for natural products and synthetic compounds that bind to and/or modulate KCNQ protein activity. A process for detecting KCNQ protein modulators requires transforming a suitable vector into compatible host cells as described previously herein. One treats such transformed cells with test substances (e.g.,

synthetic compounds or natural products), and then measures activity in the presence and absence of the test substance.

Gene Therapy

Persons skilled in the art can also use sense and antisense nucleic acid molecules as therapeutic agents for KCNQ-related indications. One may construct vectors that direct the synthesis of the desired DNA or RNA or formulate the nucleic acid as described in the art.

Several references describe the usefulness of antisense molecule. See Toulme and Helene (1988), Gene 72: 51-8; Inouye (1988), Gene, 72: 25-34; Uhlmann and Peyman (1990), Chemical Reviews 90: 543-584; Biotechnology Newswatch (January 15, 1996), p. 4; Robertson, Nature Biotechnology 15: 209 (1997); Gibbons and Dzau (1996), Science 272: 689-93. One can design them based on genomic DNA and/or cDNA, 5' and 3' flanking control regions, other flanking sequences, intron sequences, and nonclassic Watson and Crick base pairing sequences used in formation of triplex DNA. Such antisense molecules include antisense oligodeoxyribonucleotides, oligoribonucleotides, oligonucleotide analogues, and the like, and may comprise at least about 15 to 25 bases.

Antisense molecules may bind noncovalently or covalently to the KCNQ DNA or RNA. Such binding could, for example, cleave or facilitate cleavage of KCNQ DNA or RNA, increase degradation of nuclear or cytoplasmic mRNA, or inhibit transcription, translation, binding of transactivating factors, or pre-mRNA splicing or processing. Antisense molecules may also contain additional functionalities that increase stability, transport into and out of cells, binding affinity, cleavage of the target molecule, and the like. All of these effects would decrease expression of KCNQ protein and thus make the antisense molecules useful as KCNQ protein modulators.

Detailed Description of Preferred Embodiments

Human KCNQ2 and KCNQ3

Genetic properties

KCNQ1-Related (KCNQ2/KCNQ3) expressed sequence tags (ESTs) were discovered by a GCG BLAST search of the GenBank database with KCNQ1 sequence. Primers, derived from the consensus sequences of EST clones, were used to amplify human brain-derived cDNA and 877 bp and 325 bp fragments were isolated for KCNQ2 and KCNQ3, respectively. (Figure 1, probe I). To obtain full-length cDNA sequences of both genes, we employed 5'RACE PCR, screening of cDNA libraries, and Gene Trapper techniques. The composite full-length cDNAs of KCNQ2 (SEQ ID NO:3) and KCNQ3 (SEQ ID NO:17) contain an open reading frame (ORF) encoding an 871 (SEQ ID NO:4) and 854 (SEQ ID NO:18) amino acid polypeptide, respectively (Figure 2 and Figure 23). DNA sequence analysis and conceptual translation of both cDNAs reveals that they encode proteins with the structural features of a voltage-gated potassium channel and are most closely related to KCNQ1. Sanguinetti et al. (1996), Nature 384: 80-83; Yang et al. (1997), Proc. Natl. Acad. Sci. USA 94:4017-2. KCNQ2 exhibits a high degree of sequence similarity with KCNQ3 (≈70%), indicating that they belong to the same subfamily. Both proteins have a longer C-terminal domain (~200 amino acids) than KCNQ1. The initiation codon for KCNQ2 is flanked by a consensus ribosome binding site (i.e., Kozak) ACCATGG (Figure 2).

At the amino acid level, sequence analysis reveals that KCNQ2/KvLR1 contains the GYG (i.e., Gly-Tyr-Gly) potassium channel pore "signature sequence" and, therefore, is likely to encode a potassium-selective channel. A comparison of KCNQ2 and KCNQ1 (KvLQT1) reveals that the amino acid sequence identity is approximately 60% in the transmembrane and pore regions (Figure 3). KCNQ3 exhibits about the same degree of identity (about 56%) with KCNQ1 as KCNQ2 in the transmembrane and pore regions (Figure 4). The identity in the amino-terminal and carboxy-terminal domain is much less compared to the central conserved regions (Figure 3). Such findings suggest that KCNQ2/KvLR1 and KCNQ3/KvLR2 are additional members of the KCNQ1/KvLQT1 family of ion channels.

KCNQ2- and KCNQ3-specific transcripts are detectable only in human brain (Figure 5). This expression pattern is distinct from KCNQ1/KvLQT1, which is expressed strongly in human heart and pancreas as revealed by Northern blot analysis. Sanguinetti et al. (1996) Nature 384: 80-83; Yang et al. (1997), Proc. Natl. Acad. Sci. USA 94:4017-2. Expression of human KCNQ2/KvLR1 is high in the hippocampus, caudate nucleus, and amygdala; moderate in the thalamus; and weak in the subthalamic nucleus, substantia nigra and corpus callosum (Figure 5). A separate Northern blot demonstrates that expression of human KCNQ2/KvLR1 is high in the cerebral cortex; is moderate in the putamen, temporal lobe, frontal lobe, occipital pole and cerebellum; and is low in the medulla and spinal cord (Figure 5). KCNQ3 exhibits a nearly identical expression pattern in the brain (Figure 5). In order to characterize further the cell types that express KCNQ2/KvLR1, a murine-specific KCNQ2/KvLR1 cDNA fragment was isolated and used as an in situ hybridization probe. The result (Figure 9) shows that KCNQ2/KvLR1 is expressed in the mouse hippocampus and dentate gyrus, areas that are important in learning and memory.

Electrophysiological properties

The full-length human KCNQ2 and KCNQ3 cDNAs were subcloned into a Xenopus expression vector and cRNA was generated by in vitro transcription. The properties of the channels encoded by human KCNQ2 and KCNQ3 were investigated by expressing the transcribed cRNA in Xenopus oocytes. Figure 6 compares currents recorded from oocytes that were injected 5 days earlier with either water (Figure 6A) or 14 ng of human KCNQ2/KvLR1 cRNA (Figure 6B). Oocytes injected with human KCNQ2/KvLR1 cRNA exhibited outward currents that activated at potentials positive to -60 mV and had a maximal amplitude of 1 μA at +40 mV. Similar currents were never observed in water-injected control oocytes and small leak or endogenous currents recorded in control oocytes never exceeded 0.15 mA at +40 mV. The human KCNQ2/KvLR1 currents exhibited a rapidly activating delayed rectifier current phenotype very similar to hKCNQ1/KvLQT1 current. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetti et al. (1996), Nature 384: 80-83; ; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4017-2. KCNQ2/KvLR1 current rectified weakly at positive voltages.

Although the macroscopic KCNQ2/KvLR1 and KCNQ1/KvLQT1 currents are similar, KCNQ2/KvLR1 tail currents lack the "hook" observed with KCNQ1/KvLQT1 tail current. Figure 6C shows the peak current-voltage (IV) relationship for oocytes expressing KCNQ2/KvLR1 (n = 12). The K^+ selectivity of the expressed current was examined by investigation of tail current reversal potentials in bath solutions containing 2, 10, 40 and 98 mM K^+. Reversal potentials closely followed the Nernst potential for K^+ revealing a K^+-selective channel (n = 6; Figure 6D). The reversal potential for KCNQ2/KvLR1 current shifted by 52 mV per 10-fold change in external K^+. The dashed line has a slope predicted from the Nernst equation for a perfectly selective K^+ channel.

A family of currents elicited by depolarizing voltage steps in an oocyte injected with KCNQ3 cRNA are shown in Figure 18A. The currents activate at potentials positive to -70 mV and rectify inwardly at potentials greater than 0 mV, as is obvious from the IV relationship (Figure 18B). The KCNQ3 reversal potential shifted 49 mV per 10-fold change in external K^+ (Figure 18C). Thus, although still predominantly selective for K^+, KCNQ3 is slightly less K^+-selective than KCNQ2.

Co-expression of KCNE1 (KCNE1 is also known as "minK" or "Isk") with KCNQ1/KvLQT1 dramatically alters the amplitude and gating kinetics of KCNQ1/KvLQT1 current. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetti et al. (1996), Nature 384: 80-83; ; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4017-2. MinK is a polypeptide thought to encode or regulate a K^+ channel. Folander et al. (1990) Proc. Natl. Acad. Sci. USA 87: 2975-2979; Varnum et al. (1993) Proc. Natl. Acad. Sci. USA 90: 11528-11532; Ben-Efraim et al. (1996) J. Biol. Chem. 271: 8768-8771. These studies suggest that minK and KCNQ1/KvLQT1 co-assemble to form the K^+ channel underlying the slow delayed rectifier current in heart. A similar association between minK and KCNQ2/KvLR1 was tested. Coexpression of KCNE1 with KCNQ2/KvLR1 had little effect on the KCNQ2/KvLR1 current in oocytes, and separate currents carried by KCNQ1/KvLQT1 and KCNQ2/KvLR1 channels could be delineated in oocytes co-injected with minK and KCNQ2/KvLR1 using selective inhibitors for each of the channels. Thus, KCNQ2/KvLR1 interacts

differently with KCNE1 than does KCNQ1/KvLQT1. Different KCNQ members may functionally interact with proteins structurally similar to KCNE1.

Pharmacological properties

Inhibitors of various potassium channels present in brain and other tissues were used to investigate the pharmacology of KCNQ2/KvLR1. The effects of 0.2 mM of 4-aminopyridine (4-AP), 10 μM E-4031, 10 μM clofilium, 0.1 mM of charybdotoxin, and 1 mM tetraethylammonium (TEA) on KCNQ2/KvLR1 currents recorded from a single oocyte are shown in Figure 7. Each of these compounds was also tested alone in individual oocytes and the effects of each agent were no different.

Charybdotoxin is a scorpion venom protein that inhibits a variety of Ca^{2+}-activated and voltage-dependent K^+ channels. Miller et al. (1985), Nature 313: 316-8; Sugg et al. (1990), J. Biol. Chem. 265: 18745-8. Charybdotoxin did not inhibit the KCNQ2/KvLR1 current at the concentration tested. This toxin also had no effect on KCNQ1/KvLQT1.

E-4031 (10 mM) is a selective inhibitor of I_{Kr}. Sanguinetti et al. (1990) J. Gen. Physiol. 96: 195-215). 4-AP (0.2 mM) is an inhibitor of Shaker-type K^+ channels. Deal et al. (1996) Physiol. Rev. 76: 49-67. Neither E-4031 nor 4-AP produced significant effects on KCNQ2/KvLR1 current. Similarly, both reagents do not inhibit KCNQ1/KvLQT1 currents. Yang et al. (1997), Proc. Natl. Acad. Sci. USA 94:4017-21.

TEA is a weak inhibitor of KCNQ1/KvLQT1 whereas clofilium is a strong inhibitor of KCNQ1/KvLQT1. Yang et al. (1997), Proc. Natl. Acad. Sci. USA 94:. Clofilium also inhibits cardiac I_{Kr} and I_{Ks}. Arena et al. (1988), Molecular Pharmacology 34: 60-66; Colatsky et al. (1990), Circulation 82: 2235-42. For KCNQ2/KvLR1, in contrast, clofilium had little effect whereas TEA inhibited the current by over 85% at a concentration of 1 mM.

The pharmacology of KCNQ3 was significantly different than that of KCNQ2 (Figure 18D). Clofilium (10 μM) reduced KCNQ3 current by 30% from control but had little effect on KCNQ2. TEA, which strongly inhibited KCNQ2 at 1 mM, produced little inhibition of KCNQ3 at 5mM. CTX (100 nM), 4-AP (2 mM) and E-4031 (10 μM) also had no effect on KCNQ3 current.

As can be seen from these results, the pharmacological properties of KCNQ3/KvLR2, KCNQ2/KvLR1 and KCNQ1/KvLQT1 are quite different.

KCNQ2 and KCNQ3 functionally interact

The overlapping expression pattern of KCNQ2 and KCNQ3 in different brain regions (Figure 5), prompted us to test for functional interaction between the two channels. Families of currents elicited by depolarizing voltage steps in oocytes injected with KCNQ2 and KCNQ3 alone and together are shown in Figure 19A through Figure 19C. Current amplitudes recorded from oocytes co-expressing the two channels were 15-fold greater than in oocytes injected with each of the channels individually. Peak current amplitudes at +30 mV for KCNQ2, KCNQ3 and KCNQ2+KCNQ3 were 0.98±0.09 (n=6), 0.98±0.06 (n=5) and 14.2±0.62 μM (n=6), respectively. Quantitatively similar results were obtained in 3 separate batches of oocytes. The IV relationship shows that KCNQ2+KCNQ3 currents activated at potentials positive to -60 mV and did not rectify, unlike KCNQ2 and particularly KCNQ3, at positive voltages (Figure 19D). The reversal potential of tail currents shifted by 57 mV per 10-fold change in external K^+ indicating that KCNQ2+KCNQ3 is nearly perfectly selective for K^+ (Figure 19E). KCNQ2+KCNQ3 current is weakly sensitive to inhibition by 5 mM TEA and 10 μM clofilium but not to 100 nM CTX or 2 mM 4-AP (Figure 19F). E-4031 (10 μM) also did not inhibit KCNQ2+KCNQ3 current (not shown). These results suggest strongly that KCNQ2+KCNQ3 interact to form a channel with properties distinct from either KCNQ2 or KCNQ3 channels alone.

KCNE1 interacts with KCNQ2+KCNQ3 channels

The β subunit KCNE1 dramatically alters the amplitude and gating kinetics of the KCNQ1 channel. Barhanin et al. (1996) Nature 384: 78-80; Sanguinetti et al. (1996), Nature 384: 80-83; Yang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4017-2; Romey et al. (1997) J. Biol. Chem. 272:16713-16716. Because KCNQ2 and KCNQ3 are members of the same K^+ channel subfamily, we tested for an interaction between KCNE1 and KCNQ2+KCNQ3 channels. Figure 20 shows currents elicited by 1 sec depolarizing voltage steps in oocytes expressing KCNE1 alone (Figure 20A),

KCNQ2+KCNQ3 (Figure 20B), and KCNQ2+KCNQ3+KCNE1 (Figure 20C). KCNE1 significantly attenuated KCNQ2+KCNQ3 current amplitude and slowed gating kinetics. Peak current amplitude at +30 mV was reduced by 62±6.0 % (n=6) in oocytes co-expressing KCNE1. Activating currents were fitted to a bi-exponential function to determine fast and slow time constants of activation. Fast and slow time constants for activation of KCNQ2+KCNQ3 current at +10 mV were 50.1±3.4 (n=6) and 239.3±17.5 ms (n=6), respectively; these were shifted to 124.7±8.8 (n=5) and 680.7±71.4 ms (n=6) when KNCE1 was injected together with KCNQ2+KCNQ3. Similar results were obtained in more than 15 oocytes from each group in this and two additional batches of oocytes. KCNE1 currents appear absent because of the duration (1 sec) of the voltage steps used and the scale at which the currents are shown. However, as shown clearly in the inset in Figure 20A, 5 sec voltage steps elicited typical KCNE1 currents in the same oocyte. The effect of KCNE1 on gating kinetics is similar for KCNQ1 and KCNQ2+KCNQ3 channels. In contrast, KCNE1 augments KCNQ1 current but inhibits KCNQ2+KCNQ3.

The results explain why mutations in either of two unlinked K^+-channel encoding genes yield the same phenotype. BFNC-associated mutations in either KCNQ2 or KCNQ3 could cause a profound reduction in KCNQ2+KCNQ3 current amplitude. One study has shown that a BFNC-causing mutation resulting in an nonfunctional, truncated KCNQ2 protein, failed to produce a dominant-negative inhibition of wild-type KCNQ2 channels expressed in oocytes. Biervert et al. (1998), Science 279:403-406. The present invention, demonstrating a synergistic interaction between KCNQ2 and KCNQ3, may provide a likely explanation for this finding. That is, mutations in KCNQ2 may only produce dominant-negative effects when co-expressed with wild-type KCNQ3 channels, and vice versa.

Molecular genetics

Recent advances in molecular genetics has allowed us to correlate potassium channels with diseases in the nervous system. Most recently, and as discussed above, BFNC, a class of idiopathic generalized epilepsy, was recently

linked to mutations in KCNQ2 and KCNQ3. Biervert et al., supra; Charlier et al., supra; and Singh et al., supra. The identification and expression of human KCNQ2 and human KCNQ3 will allow us to investigate further correlations with BFNC and other potential human disease. The present invention will now permit those skilled in the art to identify modulators, e.g., activators, of KCNQ2 and/or KCNQ3. Modulators of KCNQ2 and/or KCNQ3 may provide opportunity for treatment of disease, such as BFNC. Additionally, because human KCNQ2 and KCNQ3 are expressed highly in areas associated with learning and memory, modulators of KCNQ2 and/or KCNQ3 may also provide opportunity for pharmacological treatment of the memory loss associated with advanced age, Parkinson's disease or Alzheimer's disease.

Murine KvLR1

Starting with a brain expressed sequence tag (EST, public domain database) similar to the KvLQT1 gene, a novel potassium channel gene was cloned from a mouse brain library and functionally expressed. Figure 10A through Figure 10D shows the murine KCNQ2/KvLR1 gene (SEQ ID NO:5) encoding for a protein of 722 amino acids (SEQ ID NO:6) and a calculated molecular weight of 80.4 kDa. Hydropathy analysis (Figure 10E) illustrates the computer-generated topology of KvLR1 to have 6 membrane spanning domains and a pore domain typical of voltage-gated potassium channels.

The amino acid alignment of the murine KCNQ2/KvLR1 channel with the murine KCNQ1/KvLQT1 channel is shown in Figure 11. Overall, there is 40% identity between the two channels with 62.5% identity within the spanning and pore domains. Phylogenetic analysis suggests that the murine KCNQ2/KvLR1 gene to be a member of the KCNQ1/KvLQT1 gene family and to be distantly related to the HERG gene and other voltage-gated family members. Signature amino acid sequences characteristic of voltage-gated potassium channels are present within murine KCNQ2/KvLR1; a repeating arginine pattern is seen within the S4 spanning domain known as the voltage sensor, and a GYG sequence within the pore region. Further analysis of several 3' RACE clones indicate diversity past the S6 membrane

spanning domain. To date, two alternative splice exons, A and B, have been identified, the amino acid sequences of which are shown in Figure 12.

To determine tissue distribution of murine KCNQ2/KvLR1, a northern blot was performed with a probe from the murine KCNQ2/KvLR1 channel that did not contain the pore or voltage sensor regions. This sequence of the gene avoids possible cross-reactivity with other channels. The results, shown in Figure 13, indicate a highly abundant 8.2 kb message found only in the brain and not observed in peripheral tissues. Although not absolute, longer exposures of the northern blot did not indicate the presence of the message in the peripheral tissues indicated in Figure 5.

To obtain higher resolution of message localization within the brain, in situ hybridization was performed. Positive hybridization signal with an antisense riboprobe specific for a nonconserved region of the KCNQ2/KvLR1 gene is observed with a broad distribution throughout much of the rat brain. The mouse probe was 99% identical to the rat sequence. Robust signal, however, is observed with a more limited distribution in the following regions: piriform cortex, supraoptic nucleus, amygdala, hippocampus, including the CA1, 2, and 3 regions and the dentate gyrus, MO5 (motor nucleus of the brain stem trigeminal), facial nucleus, hypoglossal nucleus, inferior olivary nuclei, deep cerebellar nuclei, gigantocellular nuclei, lateral and medial vestibular nuclei, motor neurons of the spinal cord, and sensory neurons of the dorsal root ganglion. Moderate levels of hybridization signal are also observed in the cortex, septum, striatum, hypothalamus, thalamus, medial habenula, substantia nigra compacta, mammillary nuclei, lateral and medial geniculate, interfasicular nucleus, purkinje and granule cells of the cerebellum, parabrachial nuclei, dorsal and ventral cochlear nuclei, and other brain stem nuclei. A composite view of three regions is shown in Figure 14.

To test for functional expression, cRNA was prepared from the murine KCNQ2/KvLR1 gene and injected into Xenopus oocytes. In a two-electrode voltage clamp, a family of outward currents were generated in murine KCNQ2/KvLR1 cRNA-injected oocytes (n>20). After a minimum of 48 hours, currents qualitatively and quantitatively different than native currents generated with identical protocols in water injected or uninjected control cells (representing Ca^{2+} -activated chloride

currents and other native currents) (Figure 15). The murine KCNQ2/KvLR1-mediated currents were blocked by 1 mM TEA. Similar currents were obtained from CHO cells stably expressing murine KCNQ2 and recorded using patch-clamp techniques. Single channel conductances were estimated to be 24-30 pS in symmetrical 140 mM potassium. (Figure 22).

To determine if murine KCNQ2/KvLR1 has similar pharmacology to I_{Ks} and I_{Kr} currents in cardiac myocytes, clofilium was tested on oocytes expressing murine KCNQ2. At 20 μM, clofilium was shown to partially block the murine KCNQ2-mediated currents. Other specific K^+ channel blocking toxins, including iberiotoxin, α-dendrotoxin and charybdotoxin, had no significant effect on murine KCNQ2-mediated currents.

Materials and Methods

Human KCNQ2

Molecular cloning and expression of human KCNQ2 (human KvLR1) and human KCNQ3 (human KvLR2)

5' RACE PCR was performed by amplifying human brain or fetal brain cDNA libraries or Marathon-Ready cDNAs (Clontech) using primers derived from the KvLQT1-related EST sequences (EST# yn72g11, yo31c08, ys93a07 (sequences can be found in Genbank database)) (Figure 1). PCR products were gel-purified, subcloned and sequenced. Random-primed ^{32}P-labelled DNA probes containing specific regions of KCNQ2 or KCNQ3 sequence were used for screening of cDNA libraries and Northern blot analysis using standard protocol. For example, KCNQ2 Probe I (Figure 1) was used for Northern blot analysis; Probe II (Figure 1) was used for screening human brain cDNA libraries according to standard protocols.

The Gene Trapper experiment was performed using the protocol as described in the Manufacturer's manual (LifeTechnologies). The composite full-length human KCNQ2 and human KCNQ3 cDNA clones were obtained by restriction enzyme digestion and ligation of overlapping cDNA clones. The full-length cDNAs were subcloned into a Xenopus expression vector, derived from pSP64T plasmid.

Capped cRNA for microinjection was synthesized using mMESSAGE mMACHINE Kit (Ambion).

For detection of expression of KCNQ2 as shown in Figures 9A and 9B, tissue processing, histological analyses and in situ hybridization analyses were performed essentially as described in Fagan et al. (1996), J. Neurosci. 16 (19): 6208-18.

Electrophysiological and pharmacological characterization of KCNQ2 and KCNQ3

Stage V and VI Xenopus laevis oocytes were defolliculated with collagenase treatment and injected with cRNAs, as described in Yang et al., supra. Currents were recorded at room temperature using the two-microelectrode voltage clamp (Dagan TEV-200) technique between 3-5 days after injection of KCNQ2 (15 ng), KCNQ3 (15 ng), or KCNE1 (2 ng) cRNA alone or in combination. Microelectrodes (0.8 to 1.5 MΩ) were filled with 3 M KCl. Bath solution contained (in mM): 96 NaCl, 2 KCl, 0.4-1.8 $CaCl_2$, 1-2 $MgCl_2$ and 5 HEPES (pH 7.5). KCl was varied in some experiments by equimolar substitution with NaCl.

K^+ selectivity was assessed by determining the dependence of tail current reversal potential on the external K^+ concentration. Tail currents were elicited at potentials of -110 to +10 mV following a voltage step to +20 mV while the external K^+ concentration was varied between 2, 10, 40, and 98 mM. Current reversal potential under each condition was determined for each oocyte by measuring the zero intercept from a plot of tail current amplitude vs test potential.

Axoclamp (Axon Instruments) was used for generating voltage clamp commands and acquiring data and Axograph 3.0 (Axon Instruments) was used for data analysis. All data was sampled at rates at least two times the low pass filter rate. Experiments were performed at 22-25 °C. Clofilum was obtained from RBI Biochemicals and 4-aminopyridine (4-AP), TEA and charybdotoxin were obtained from Sigma Chemical Co. E-4031 was synthesized from information published by Esai Research Laboratories.

Murine KvLR1

Probe preparation and library screening

A unique expressed sequence tag (EST) was identified from the public database that has similarity to the KvLQT gene. Oligonucleotide primers were synthesized from the EST sequence for PCR experiments. The forward primer (SEQ. ID. NO.: 15) was

5'-GAG TAT GAG AAG AGC TCG GA-3'

and reverse primer (SEQ. ID. NO.: 16) was

5'-CAG ATG TGG CAA AGA CGT TGC-3'.

Rat brain polyA$^+$ RNA was reverse-transcribed with random hexamers and amplified by PCR [60 sec 94 °C, 90 sec 55 °C, 120 sec 72 °C, 30 cycles] with the above primers. A 240 bp DNA fragment of rat KCNQ2/KvLR1 was isolated by gel electrophoresis and subcloned into pCRII (InVitrogen). The 240 bp DNA fragment was random-prime labeled with ^{32}P-dCTP and used as a probe to screen a mouse brain pcDNA1 plasmid library (Clontech, Palo Alto, CA). Overall, 2x10^5 colonies were screened using standard filter lift protocols. The filters were hybridized overnight in 50% formamide, 2X PIPES and 1% SDS at 42 °C and washed 1x in 1X SSC then 3x 20 minutes in 0.1x SSC, 0.1% SDS at 53 °C. Filters were exposed overnight at -70 °C. Only one positive colony was identified and replated until purified. Clone mbr 26.1, designated murine KvLR1, was sequenced on both strands by dideoxy termination reactions.

Northern Blots

Northern blots were performed with the mouse multiple tissue blot (Clontech) according to the manufacturer's instructions. Briefly, the blot was prehybridized at 68°C with ExpressHyb solution for 30 minutes. A DNA fragment was isolated from the murine KvLR1 coding region by the restriction enzyme PvuII, which eliminated the pore and voltage sensor consensus sequences, and random-prime labeled with ^{32}P-dCTP, denatured at 100°C for 5 minutes, chilled on ice and added to fresh ExpressHyb before addition to the northern blot. The blot was incubated for 60 minutes at 68°C with continuous shaking. The blot was washed 2X at 50°C in 0.1X SSC and 0.1% SDS. The blot was wrapped in saran wrap and exposed to x-ray film

overnight at room temperature. The same protocol was used for the actin probe provided with the blot.

In situ hybridization

Frozen sections cut at intervals of 225 μm through the entire adult rat brain were fixed by immersion (without thawing) into ice cold 10% formaldehyde in PBS for 20 minutes and rinsed with PBS. Fixed sections of rat DRG were treated with 0.5% Triton X-100 in 0.1 M Tris, pH 8.0, and 0.05 M EDTA for 30 minutes and rinsed for 3 minutes in 0.1 M Tris, pH 8.0, and 0.05 M EDTA. The tissue was then treated with 0.1 M TEA, pH 8.0, plus 0.25% acetic anhydride for 10 minutes at room temperature, rinsed (3 X) in 2X SSC, dehydrated through a series of alcohols, delipidated in chloroform, and air dried.

Riboprobes were synthesized using the Promega Riboprobe Transcription System II with 250 μCi ^{35}S-UTP and 250 μCi ^{35}S-CTP in a total reaction volume of 10 μL. Unlabeled UTP and CTP were added at 25 μM each and ATP and GTP at 500 μM each. The murine KCNQ2/KvLR1 plasmid (nts 552-1125 subcloned into pBluescript II) was linearized with Sac I and transcribed using T3 RNA polymerase, and with BamHI and transcribed using SP6 RNA polymerase to generate anti-sense and sense probes, respectively. One μg of linearized plasmid was added for each reaction. The riboprobes were purified by phenol:chloroform extraction and two ethanol precipitations using ammonium acetate. The dried tissue sections were hybridized with 1 X 10^7 cpm/ml riboprobe in hybridization buffer (50% formamide, 0.3 M NaCl, 10 mM Tris, 1 mM EDTA, 1X Denhardt's solution, 10% dextran sulfate, 500 μg/ml tRNA and 10 mM DTT) overnight at 55°C. The hybridization solution was removed by rinsing 4 times in 4X SSC, 5 minutes for each wash. The sections were incubated in 0.02 mg/ml RNase, 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA for 30 minutes at 37°C, then washed in 2X SSC, 1X SSC and 0.5X SSC, all containing 1 mM DTT, for 10 minutes per wash at room temperature. The tissues were incubated in 0.1X SSC, 1 mM DTT for 30 minutes at 55°C, then rinsed briefly in 0.1X SSC and 1 mM DTT at room temperature, dehydrated, and air dried. The dried sections were exposed to XOMAT film (Kodak, Rochester, NY), then were

dipped in NTB2 emulsion (Kodak, Rochester, NY) to determine the cellular localization of each mRNA.

Expression and Recording in Oocytes

The murine KCNQ2/KvLR1 cDNAs were linearized with the restriction enzyme NotI and in vitro transcribed using the mMessage mMachine T7 RNA polymerase kit according to the manufacturer's instructions (Ambion, Austin, TX). The cRNAs were solubilized in RNase-free water, and stored at –70 °C at a concentration of 1.0 μg/μl. Frog oocytes were prepared and injected using standard techniques (Colman, 1984). In murine KvLR1 expression experiments, each oocyte was injected with approximately 35-40 nl of the cRNA. Following injection, oocytes were maintained at 17°C in ND96 medium consisting of (in mM): NaCl, 90; KCl, 1.0; $CaCl_2$, 1.0; $MgCl_2$, 1.0; HEPES, 5.0; pH 7.5. Horse serum and penicillin/streptomycin, both 5% of final volume, were added as supplements to the incubation medium. Electrophysiological recording commenced 2-6 days following cRNA injection. Prior to the start of an experiment oocytes were placed in a recording chamber and incubated in Modified Barth's Solution (MBS) consisting of (in mM): NaCl, 88; $NaHCO_3$, 2.4; KCl, 1.0; HEPES, 10; $MgSO_4$, 0.82; $Ca(NO_3)_2$, 0.33; $CaCl_2$, 0.41; pH 7.5. Oocytes were impaled with electrodes (1-2 MΩ) and standard 2-electrode voltage clamp techniques were employed to record whole-cell membrane currents (Stuhmer, 1992; TEC 200, Dagan Instruments). Voltage-clamp protocols typically consisted of a series of voltage steps 100-500 ms duration, in +10 mV steps from a holding potential of -60 mV to -90 mV to a maximal potential of +40 mV to +50 mV; records were digitized at 5 kHz and stored on a computer using pClamp 6.0 software (Axon Instruments), and analyzed using ClampFit or AxoGraph software (Axon Instruments).

Expression and Recording in CHO cells

Patch clamp recordings were obtained from CHO cells that transiently or stably expressed murine KCNQ2 channels. Electrodes were prepared using a PC-84 Sachs-Flaming pipette puller (Sutter Instruments) and fire-polished to a final tip resistance of 3-5 MΩ. Pipettes were filled with a solution that consisted of (in mM)

KCl (140), MOPS (20), K_2EGTA (1.0), $CaCl_2$ (0.89), pH 7.2. The pipette solution sometimes contained $MgCl_2$ (1.0) to aid in seal formation. Cells were grown on poly-D-lysine coated coverslips, and pieces of the coverslips containing CHO cells were placed into a chamber on an inverted microscope for recording. Prior to recording, and during seal formation, cells were bathed in an external solution consisting of (in mM) NaCl (145), KCl (3), $CaCl_2$ (2.5), $MgCl_2$ (1.0), HEPES (10), pH 7.4. Electrodes were lowered to the surface of cells under visual inspection; following gigaseal formation inside-out membrane patches were excised into an internal solution consisting of (in mM) KCl (140), MOPS (20), K_2EGTA (1.0), $CaCl_2$ (0.89), pH 7.2. All recordings were made under symmetrical K^+ conditions. Following patch excision continuous and step-protocol voltage-clamp recordings were obtained, and analyses performed, using an AxoPatch 200B Patch Clamp amplifier and pClamp software (Axon Instruments). Results are shown in Figure 22.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

What is claimed is:

1. An isolated nucleic acid molecule encoding a polypeptide comprising all or a portion of a KCNQ protein, wherein said KCNQ protein is selected from the group consisting of human KCNQ2 protein, human KCNQ3 protein, murine KCNQ2 protein, and rat KCNQ2 protein.

2. The nucleic acid molecule of claim 1 wherein said human KCNQ2 protein comprises the amino acid sequence as shown in SEQ ID NO:4.

3. The nucleic acid molecule of claim 1 wherein said human KCNQ3 protein comprises the amino acid sequence as shown in SEQ ID NO:18.

4. The nucleic acid molecule of claim 1 wherein said murine KCNQ2 protein comprises the amino acid sequence as shown in SEQ ID NO:6.

5. The nucleic acid molecule of claim 1 selected from the group consisting of: (a) all or a portion of a nucleic acid sequence as shown in SEQ ID NO:3; (b) the complement of (a); and (c) variations of (a) due to degeneracy in the genetic code.

6. The nucleic acid molecule of claim 1 selected from the group consisting of: (a) all or a portion of a nucleic acid sequence as shown in SEQ ID NO:17; (b) the complement of (a); and (c) variations of (a) due to degeneracy in the genetic code.

7. The nucleic acid molecule of claim 1 selected from the group consisting of: (a) all or a portion of a nucleic acid sequence as shown in SEQ ID NO:5; (b) the complement of (a); and (c) variations of (a) due to degeneracy in the genetic code.

8. A vector comprising the nucleic acid molecule of claim 1.

9. A vector comprising the nucleic acid molecule of claim 2.

10. A vector comprising the nucleic acid molecule of claim 3.

11. A vector comprising the nucleic acid molecule of claim 5.

12. A vector comprising the nucleic acid molecule of claim 6.

13. A prokaryotic or eukaryotic host cell comprising the vector of claim 8.

14. A prokaryotic or eukaryotic host cell comprising the vector of claim 9.

15. A prokaryotic or eukaryotic host cell comprising the vector of claim 10.

16. A prokaryotic or eukaryotic host cell comprising the vector of claim 11.

17. A prokaryotic or eukaryotic host cell comprising the vector of claim 12.

18. An isolated KCNQ protein or polypeptide comprising all or a portion of an amino acid sequence selected from the group consisting of human KCNQ2 as shown in SEQ ID NO:4, human KCNQ3 as shown in SEQ ID NO:18, murine KCNQ2 as shown in SEQ ID NO:6, and rat KCNQ2 as shown in SEQ ID NO:8.

19. An isolated nucleic acid molecule selected from the group consisting of: (a) a nucleotide sequence that exhibits at least 80% sequence identity to the nucleotide sequence as shown in SEQ ID NO:3; (b) a nucleotide sequence that exhibits at least 80% sequence identity to the nucleotide sequence as shown in SEQ ID NO:17; and (c) a nucleotide sequence that exhibits at least 80% sequence identity to the nucleotide sequence as shown in SEQ ID NO:5.

20. A method of screening for a compound that is capable of modulating the activity of a KCNQ protein, comprising the steps of:

(a) providing a host cell of claim 13;

(b) determining the activity of said KCNQ protein in the absence of said compound;

(c) contacting the cell with said compound; and

(d) determining the activity of said KCNQ protein in the presence of said compound,

wherein a difference between the activity of said KCNQ protein in the presence of said compound and in the absence of said compound indicates a modulating compound.

21. An anitbody specific for the KCNQ protein of claim 18.

22. The antibody of claim 21, wherein said antibody is a monoclonal antibody.